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LipidQuan-R for Triglycerides in Human Serum: A Rapid, Targeted UPLC-MS/MS Method for Lipidomic Research Studies

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APPLICATION BENEFITS

- Simultaneous, targeted UPLC-MS/MS analysis of 54+ triglycerides (687 MRM transitions) in a single analytical run that is under eleven minutes
- High throughput analysis means larger sample sets can be analyzed
- Use of Multiple Reaction Monitoring (MRM) gives structural information for triglycerides detected
- Use of a generic LC-MS configuration yields versatility for switching from one compound class to another

WATERS SOLUTIONS

CORTECS™ UPLC™ Columns

ACQUITY™ UPLC I-Class System

Xevo™ TQ-S micro

MassLynx™ Software

TargetLynx™ Software

Quanpedia™ Software

LipidQuan-R™

KEYWORDS

Targeted, triglycerides, UPLC, tandem quadrupole, Xevo TQ-S micro, Multiple Reaction Monitoring (MRM), lipids, human serum, lipidomics

INTRODUCTION

Triglycerides (TAG's) are lipid molecules made up of a glycerol backbone and three fatty acids, connected via ester linkages. They are the most abundant lipids in human serum and are the storage form for fatty acids. Historically, triglycerides have been measured as a single combined level of all triglycerides. However, this class of compound has huge variety in terms of the triglyceride species present. This variety is due to the three different fatty acid residues that make up a triglyceride, the number of carbon atoms (NC), and the number of double bonds (DB), vary from one triglyeride to another. Furthermore, a given triglyeride (NC:DB) might have various structural isomers due to a different combination of fatty acids giving the same NC:DB combination. Here we demonstrate a high-throughput UPLC-MS/MS research method for the semi-quantitative analysis of various triglycerides in human serum samples. This method is capable of measuring 54 triglyceride NC:DB combinations and in excess of 100 individual triglycerides. This application note is also part of a LipidQuan-R Method Package.

EXPERIMENTAL

Human serum sample preparation

Human serum was protein precipitated with propan-2-ol at a ratio of 4:1 propan-2-ol:serum. This was then centrifuged for three minutes at 25,000 g. The resulting supernatant was then diluted 1:1 using deionized water and mixed. 2 µL of this was then injected onto the UPLC-MS/MS system.

LC conditions

UPLC separation was performed with an ACQUITY UPLC I-Class System (fixed loop), equipped with a CORTECS T3 2.7 µm (2.1 × 30 mm) analytical column. A sample of 2 µL was injected at a flow rate of 0.25 mL/min. Mobile phase A was 0.01% formic acid $_{(aq)}$ containing 0.2 mM Ammonium Formate and mobile phase B was 50% isopropanol in acetonitrile containing 0.01% formic acid and 0.2 mM Ammonium Formate. After an initial two minute hold at 90% Mobile phase B, the triglycerides were eluted from the column and separated with a gradient of 90–98% mobile phase B over 4 minutes, followed by a two minute column wash at 98% Mobile phase B. The column was then re-equilibrated to initial conditions. The analytical column temperature was maintained at 60 °C.

MS conditions

Multiple Reaction Monitoring (MRM) analyses were performed using a Xevo TQ-S micro Mass Spectrometer. All experiments were performed in positive electrospray ionization (ESI+) mode. The ion source temperature and capillary voltage were kept constant at 150 °C and 2.0 kV respectively. The cone gas flow rate was 50 L/hr and desolvation temperature was 650 °C. All MRM transitions were monitored at a cone voltage of 35V and a collision energy of 20 eV.

Informatics

Method information was imported onto the LC-MS system using the Quanpedia functionality within MassLynx.
This extendable and searchable database produces LC and MS methods as well as processing methods for use in TargetLynx for compound quantification.

RESULTS AND DISCUSSION

Triglycerides (TAG's) were detected using a series of MRM transitions where their precursor mass was the corresponding ammonium adduct [M+NH_4^+], and the product ions were the neutral losses of one of the three fatty acid residues and the ammonium ion. An example is given in Figure 1.

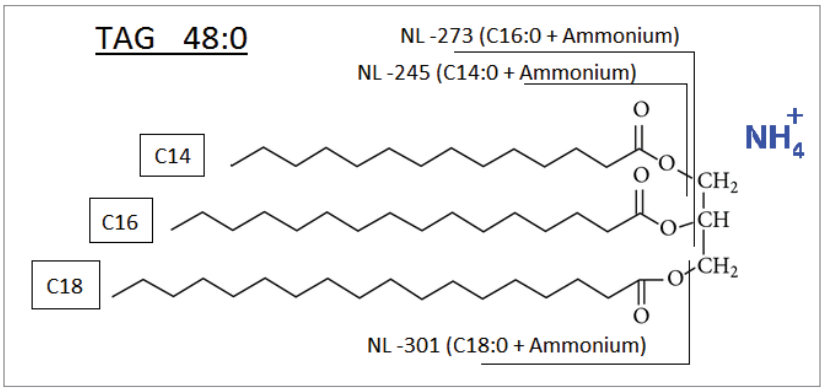

Figure 1. Fragmentation of the ammonium adduct for a given Triglyceride (TAG 48:0 [14:0/16:0/18:0]).

The precursor mass [M+NH_4^+] was calculated for each triglyceride as follows:

Precursor for TAG [NC_{TAG}:DB_{TAG}] = [(NC_{TAG} - 6) × 14] - [DB_{TAG} × 2] + 236.55

Where:

NC_{TAG} = Number of Carbon atoms in TAG (combined from the 3 fatty acids)

and

DB_{TAG} = Number of Double Bonds in TAG (combined from the 3 fatty acids)

The product ion mass for the loss of a given fatty acid (FA) from a given TAG was calculated as follows:

Product for TAG [NC_{TAG}:DB_{TAG}] - FA [NC_{FA}:DB_{FA}] = Precursor for TAG (See Above) – (Mass of FA + 18)

Where:

NC_{TAG} = Number of Carbon atoms in TAG (combined from the 3 fatty acids)

DB_{TAG} = Number of Double Bonds in TAG (combined from the 3 fatty acids)

NC_{FA} = Number of Carbon atoms in FA

DB_{FA} = Number of Double Bonds in FA

and

Mass of FA = [(NC_{FA} - 2) × 14] - [DB_{FA} × 2] + 59.15

Table 1 indicates the MRM transitions monitored during the analysis. Each triglyceride monitored for (in terms of NC_{TAG}:DB_{TAG}) is listed along with a set of numbers. When referenced against Table 2, these numbers indicate which fatty acid chain losses were monitored for that particular TAG. This corresponded to 687 MRM transitions being monitored in total.

TAG's (NC:DB)		Number of Double Bonds (DB)								
		0	1	2	3	4	5	6	7	8
Number of Carbons (NC)	36	1–8	1–10	1–11						
	38	1–8	1–10	1–11						
	40	2–8	1–10	1–11						
	42	3–8	2–10	2–11						
	44	4–8	3–10, 12–13	3–14						
	46	5–8	4–10, 12–13	4–14	4–22	4–24				
	48	6–8	5–10, 12–13	5–14	5–22	5–20, 23–24				
	50	6–8	6–10, 12–13	6–14	6–22	6–24				
	52	6–8, 12	6–10, 12–13	6–14	6–22	6–24	6–26	6–27		
	54	6–8, 12, 17, 21	6–10, 12–13, 17–18	6–14, 17–19	6–22	6–24	6–26	6–27	6–27	
	56	6–8, 12, 17, 21	6–10, 12–13, 17–18	6–14, 17–19	6–22	6–24	6–26	6–27	6–27	6–27

Table 1. MRM transitions monitored for triglycerides in terms of fatty acid residue losses (see Table 2).

Possible fatty acid residues lost during fragmentation		
Fatty Acid number	**Name/names**	**Abbreviated name (NC:DB)**
1	Butyric acid	4:0
2	Caproic acid	6:0
3	Caprylic acid	8:0
4	Capric acid	10:0
5	Lauric acid	12:0
6	Myristic acid	14:0
7	Palmitic acid	16:0
8	Stearic acid	18:0
9	Palmitoleic acid	16:1
	Sapienic acid*	
10	Oleic acid	18:1
	Elaidic acid*	
	Vaccenic acid*	
11	Linoleic acid	18:2
	Linolelaidic acid*	
12	Arachidic acid	20:0
13	Eicosenoic acid	20:1
	Gadoleic acid*	
	Gondoic acid*	
	Paullinic acid*	
14	Eicosadienoic acid	20:2
15	Linolenic acid	18:3
	Pinolenic acid*	
16	Mead acid	20:3
	*Dihomo-γ-linolenic acid**	
	*Eicosatrienoic acid**	
17	Behenic acid	22:0
18	Erucic acid	22:1
19	Docosadienoic acid	22:2
20	Docosatrienoic acid	22:3
21	Lignoceric acid	24:0
22	Nervonic acid	24:1
23	Arachidonic acid	20:4
	*Eicosatetraenoic acid**	
24	Adrenic acid	22:4
25	Eicosapentaenoic acid	20:5
26	Sardine acid	22:5
	*Ozubondo acid**	
27	Docosahexaenoic acid	22:6

**Minor isomeric forms*

Table 2. List of fatty acid residue losses monitored for in this analysis.

This UPLC-MS/MS approach allowed structural information to be determined with regards to the makeup of the triglycerides detected. Figure 2 shows chromatograms acquired for TAG's containing an even number of carbon atoms (from 48–56) and four double bonds, where the fatty acid residue lost during fragmentation was 20:4 (Arachidonic acid). Figure 2 also shows how important chromatographic separation is for this type of analysis. For TAG's 52:4, 54:4 and 56:4 the peak due to isotopic interference from the TAG containing one more double bond is significant and, in the case of TAG 56:4, is actually greater than the response from the TAG of interest.

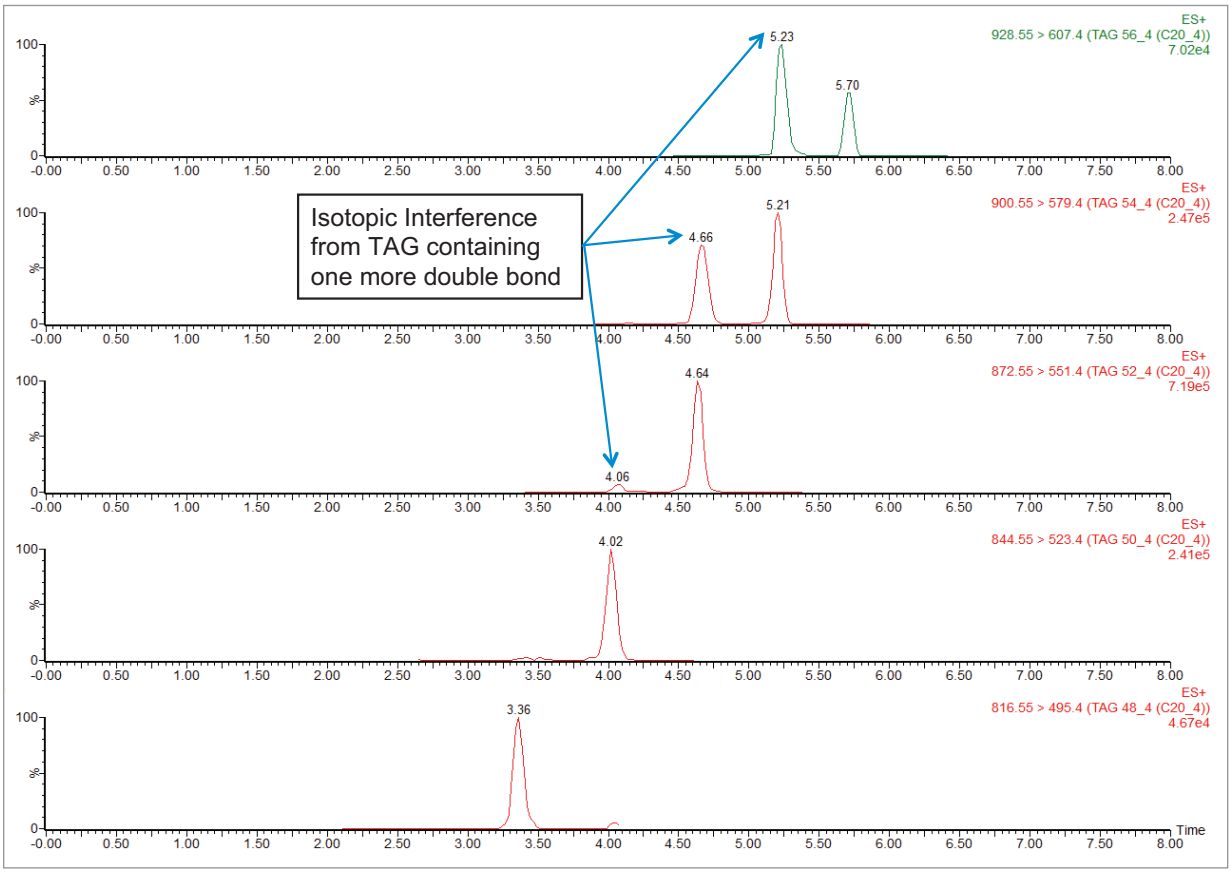

Figure 2. Chromatograms acquired for TAG's containing an even number of carbon atoms from 48–56 containing four double bonds, where the fatty acid residue lost during fragmentation was 20:4 (Arachidonic acid).

CONCLUSIONS

A rapid UPLC-MS/MS methodology has been developed for the analysis of triglycerides in biomedical research. This method has been demonstrated to be suitable for the semi-quantitative characterization of physiologically relevant levels of these important analytes in human serum. This method utilizes a single generic LC-MS platform that can be used for a variety of compound classes (including metabolomics, lipidomics, and proteomics). Deployment of this method in conjunction with other complementary LC-MS/MS Targeted Omics Method Packages available on www.waters.com can form the basis of a comprehensive suite of targeted multi-omic workflows.

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